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Diplodia bulgarica, a new record for Turkey

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Abstract—*Diplodia bulgarica* on *Malus domestica*, a new species for the Turkish mycobiota, is described briefly and illustrated. A pathogenicity test showed that *D. bulgarica* was highly aggressive to apple plants.

Key words—canker, *Botryosphaeriaceae, Dothideomycetes*

Introduction

Apple (*Malus domestica* Borkh.) is an economically important horticultural crop in Turkey; more than 3,500,000 t of apples are produced in Turkey every year, making the country the fourth largest producer worldwide (FAO 2018). Fungal pathogens are major biotic stresses affecting apple tree growth and fruit production. *Botryosphaeriaceae* encompasses a range of morphologically diverse fungi that are either pathogens, endophytes, or saprophytes, occurring mainly on woody plants such as apple (Slippers & Wingfield 2007; Phillips & al. 2012, 2013). Among the 23 genera recognized in this family is *Diplodia* (Dissanayake & al. 2016). *Diplodia* is a large genus currently comprising more than 1000 species (Phillips & al. 2012). Species of *Diplodia* can cause canker, dieback, gummosis, fruit rot, and twig blight diseases of numerous woody hosts (Crous & al. 2006, Slippers & Wingfield 2007, Lazzizera & al. 2008, Phillips & al. 2012, Abdollahzadeh 2015, Nabi & al. 2020, Hinrichs-Berger & al. 2021). Recently, a botryosphaeriaceous species associated with canker symptoms of apple trees was observed in Eğirdir district of Isparta province, Turkey. The present paper reports the etiology of the disease.

Materials & methods

In 2019, extensive canker symptoms were observed on apple trees in Eğirdir district of Isparta province, Turkey. Samples were collected from symptomatic trees showing cankers in branches and transported in a cooler to the laboratory. Fungi were isolated from canker margins after surface sterilization of samples in 2% sodium hypochlorite solution and rinsing in two changes of sterile distilled water and transferred to Petri plates (9 cm diam.) containing Potato Dextrose Agar (PDA). Plates were incubated 5–7 days at 25 °C, and the fungal isolate cultures were subculture using hyphal tips cut from the colonies under a dissecting microscope and placed, one colony per plate, onto PDA. Identification of isolates to species level was based on colony morphology and conidial characteristics, as described by Phillips & al. (2012). Isolates are maintained at the Department of Agricultural Biotechnology, Faculty of Agriculture, Aydın Adnan Menderes University, Aydın, Turkey (ADUAB).

The identity of isolates was confirmed by analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). Genomic DNA was extracted from the mycelium from the surface of the PDA cultures using an Omega Bio-Tek SP-Fungal DNA Kit according to the manufacturer's instructions. After DNA extraction of two isolates (CEE-22, CEE-211), the rDNA ITS region was amplified using primers ITS1/ITS4 (White & al. 1990). Amplicons were purified and sequenced by RefGen (Ankara, Turkey). The nucleotide sequence was compared with the GenBank nucleotide collection database through nucleotide BLAST search.

Preliminary pathogenicity tests were carried out on potted 2-year-old apple seedlings (cv. Scarlet Spur) using the representative isolates from cankers; the most virulent isolate (CEE-22) was used for greenhouse test. The outer bark at the inoculation area was disinfected by spraying with 70% ethanol, and the plants were wounded with a scalpel through the full thickness of the bark along a 1-cm long slit and inoculated with a 5 mm plug from a 7-day-old culture grown on PDA and covered with parafilm. Control seedlings were similarly wounded and plugs of sterile PDA applied. The bark of the inoculated area was removed, and the lesion lengths recorded after four weeks. Re-isolation was done from the margins of infected tissues as described above and re-isolated fungi were identified by comparing colony and conidial morphological characteristics to the original inoculated isolate (CEE-22).

Results

A total of 12 isolates were obtained by isolation from 12 cankered branches of apple trees collected from different localities of Eğirdir district of Isparta province, Turkey. Based on morphological and cultural characteristics, these isolates were tentatively identified as *Diplodia bulgarica*. The identity of two representative isolates (CEE-22 and CEE-211) was confirmed by DNA sequencing of the rDNA ITS region (GenBank accession no. MT239082 and MZ442352). The description and illustrations of *D. bulgarica* given below

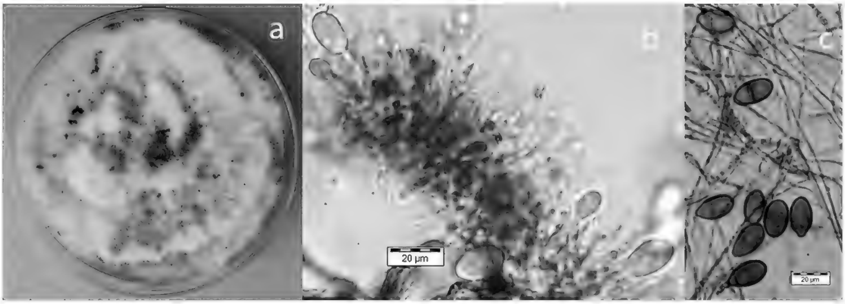

Fig. 1. *Diplodia bulgarica* (ADUAB – CEE-22): a. Colony on PDA; b. Conidia developing on conidiogenous cells; c. Septate and aseptate conidia.

are based on the Turkish collections of the material. This is the first report of *D. bulgarica* from Turkey.

Taxonomy

Diplodia bulgarica A.J.L. Phillips, J. Lopes & S.G. Bobev, Persoonia 29: 33. 2012. Fig. 1

Colony growth at 25 °C on PDA reaches 47 mm in diameter after 3 days. The colony was at first white, turned to gray to dark gray with irregular margin having dark centre and formed pycnidia after 20–25 days were immersed in culture. Pycnidia were globose to ovoid, dark brown to black. Conidia were aseptate, smooth, thick walled, oblong to ovoid, both ends broadly rounded, 19.8–29.0 × 10.4–15.1 µm, initially hyaline and later becoming pale brown and sometimes 1-septate at maturity.

Specimens examined: **TURKEY, Isparta Province, Eğirdir district**, Serpil village, from cankered branches of *Malus domestica*, 2 Nov. 2019, C. Eken CEE-22 (ADUAB; GenBank MT239082); 9 Nov. 2019, CEE-46 (ADUAB); Tepeli village, from cankered branches of *Malus domestica*, 8 Jan. 2019, C. Eken CEE-211 (ADUAB; GenBank MZ442352); 27 May 2019, CEE-212, CEE-223 (ADUAB); Balkırı village from cankered branches of *Malus domestica*, 7 Dec. 2019, C. Eken CEE-141 (ADUAB); Barla village from cankered branches of *Malus domestica*, 27 July 2019, C. Eken CEE-178, CEE-179 (ADUAB); Mahmatlar village from cankered branches of *Malus domestica*, 5 Oct. 2019, C. Eken CEE-186, CEE-191 (ADUAB); Sevinçbey village from cankered branches of *Malus domestica*, 24 Aug. 2019, C. Eken CEE-123, CEE-124 (ADUAB).

Symptoms of canker were observed on the stem of the inoculated apple seedlings. Canker lesions expanded to 52 ± 0.4 mm four weeks after inoculation and extended both upwards and downwards from the inoculation sites. The pathogen was successfully re-isolated from all inoculated stems and isolate identity was confirmed based on colony and conidial morphological characteristics. In control treatments, no symptoms developed.

Discussion

Diplodia bulgarica, originally isolated from dead twigs of *Malus sylvestris* in 2005, was described by Phillips & al. (2012). In our study, *Diplodia bulgarica* was isolated from apple trees (*M. domestica*) in the Eğirdir district of Isparta province of Turkey, showing canker symptoms consistent with previous reports (Abdollahzadeh 2015, Hanifeh & al. 2017, Bari & al. 2019, Nabi & al. 2020, Hinrichs-Berger & al. 2021).

The size of conidia of the Turkish isolates is within the ranges observed by Phillips & al. (2012; 22.5–28.0 × 14.5–18.5 µm), Hanifeh & al. (2017; 19–29 × 12.5–17.5 µm), and Nabi & al. (2020; 20.0–28.0 × 9.0–14.5 µm). The identity of isolates was confirmed by rDNA ITS sequence analysis. BLAST searches revealed that ITS sequences of Turkish isolates matched 99.82% (CEE-22) and 100% (CEE-211) with the Bulgarian ex-holotype strain of *D. bulgarica* (CBS 124136; GenBank GQ923854).

The pathogenicity of *D. bulgarica* strain CEE-22 obtained in this study was confirmed through an artificial inoculation experiment in the greenhouse. *Diplodia bulgarica* strain CEE-22 did cause canker lesions, and the strain proved to be highly aggressive. *Diplodia bulgarica* has previously been reported as an aggressive pathogen on *M. domestica* in Iran (Abdollahzadeh 2015, Hanifeh & al. 2017, Bari & al. 2019), India (Nabi & al. 2020), and Germany (Hinrichs-Berger & al. 2021). This is the first report of *D. bulgarica* associated with a disease of *M. domestica* from Turkey. This pathogen is considered to be an important agent causing canker on apple trees in Isparta province of Turkey.

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